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RESEARCH ARTICLE

Managing Western Flower Thrips (Thysanoptera: Thripidae) on Lettuce and Green Peach Aphid and Cabbage Aphid (Hemiptera: Aphididae) on Broccoli with Chemical Insecticides and the Entomopathogenic Fungus *Beauveria bassiana* (Hypocreales: Clavicipitaceae)

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Abstract:

Aims:

Lettuce and broccoli are high value vegetable crops in California. The western flower thrips, *Frankliniella occidentalis* on lettuce, and the cabbage aphid, *Brevicoryne brassicae* and the green peach aphid, *Myzus persicae* on broccoli are important insect pests that are frequently managed with chemical insecticides.

Observation:

Efficacy of various chemical insecticides and the entomopathogenic fungus *Beauveria bassiana* was evaluated against these pests in field studies in the Santa Maria area of California. Some insecticides varied in their efficacy against *F. occidentalis* from year to year and against different aphid species.

Conclusion:

A new insecticide sulfoxaflor provided good control of aphids on broccoli. *Beauveria bassiana* demonstrated a potential for broccoli and lettuce integrated pest management.

Keywords: *Beauveria bassiana*, Cabbage aphid, Green peach aphid, Western flower thrips.

1. INTRODUCTION

Lettuce and broccoli are important agricultural crops in California [1]. Lettuce is grown in 200,000 acres and is the 8th most important agricultural commodity with a crop value of $1.4 billion while broccoli, grown in 119,000, acres is ranked 17 with a value of $645 million. The western flower thrips, *Frankliniella occidentalis* (Pergande) on lettuce and the cabbage aphid, *Brevicoryne brassicae* (Linnaeus) on broccoli are important insect pests which require regular insecticidal treatments [2]. The role of *F. occidentalis* as a vector of tomato spotted wilt virus is more important than the scarring caused by its feeding. In broccoli, *B. brassicae* can stunt plant growth and even cause plant death at high infestation levels [3]. The green peach aphid, *Myzus persicae* (Sulzer) is also a frequent pest of broccoli, but is less important because it feeds mostly on older leaves. However, it requires insecticidal treatments as the presence of aphids on harvested broccoli reduces crop value.

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Among hundreds of thousands of pounds of insecticide active ingredients applied on lettuce and broccoli in 2012, entomopathogenic fungus *Beauveria bassiana* accounts for a negligible 40 lb (pounds) [4]. Repeated use of chemical insecticides can lead to the development of resistance and undermines integrated pest management (IPM) efforts. Different species of aphids and *F. occidentalis* are known to develop resistance to various insecticides around the world and the risk is especially higher for the latter [5 - 9]. Successful IPM involves rotation of insecticides with different modes of action and alternating chemicals with non-chemical options to reduce the risk of pesticide resistance. Considering entomopathogenic fungi such as *B. bassiana* can help IPM practices. *Beauveria bassiana* is pathogenic to several pests and its efficacy against *F. occidentalis* was demonstrated under greenhouse conditions in different parts of the world [10 - 13]. Pathogenicity of *B. bassiana* against different species of aphids including *M. persicae* and *B. brassicae* has also been studied under laboratory conditions [14 - 16], but information on its field efficacy against vegetable pest management especially in California is lacking.

Field studies were conducted in 2011 and 2012 in the Santa Maria area to evaluate the efficacy of existing, newly registered, and experimental insecticides. In the 2012 studies, a commercial formulation of *B. bassiana* was also evaluated. Results of these studies are important for vegetable IPM in California.

2. MATERIALS AND METHODS

2.1. *F. occidentalis* on Lettuce

Studies were conducted in commercial lettuce fields in the Santa Maria area. Each plot included a 10' long and 64" wide bed with 5 rows of lettuce and arranged in a randomized complete block design with four replications. Insecticides were applied using a CO_2-pressurized backpack sprayer equipped with three flat fan nozzles that covered the entire bed. A spray volume of 50 gallons/ac was used for all treatments except for *B. bassiana* where 100 gallons/ac were used. A non-ionic surfactant was included at 0.1% (v/v) concentration for acetamiprid, 0.125% for *B. bassiana*, and 0.25% for the rest of the treatments. The number of *F. occidentalis* was monitored before treatment and two to three times after each spray application. On each sampling date, four plants in 2011 and five plants in 2012 were randomly collected from each plot and gently beaten on a wire mesh over a plastic container with a 9X5" yellow sticky card placed inside according to the method described by Palumbo [17]. The number of *F. occidentalis* dislodged from the plants and stuck to the card were counted under a dissecting microscope. Data were summarized by the analysis of variance and significant means were separated using Tukey's HSD test.

2.1.1. 2011 Study

A lettuce field was planted in cultivar Durango on June 8. Treatments included an untreated control, acetamiprid (Assail 70 WP, 1.7 oz/ac), spinetoram (Radiant SC, 7 fl oz/ac), methomyl (Lannate SP, 0.75 lb/ac), tolfenpyrad (Torac, 21 fl oz/ac), and a combination of tolfenpyrad (Torac, 21 fl oz/ac) and methomyl (Lannate SP, 0.75 lb/ac). Treatments were administered on July 16, July 22, and August 3.

2.1.2. 2012 Study

A field was planted in cultivar Vandenberg on April 6. Treatments included an untreated control, acetamiprid (Assail 30 SD, 4 oz/ac), spinetoram (Radiant SC, 8 fl oz/ac), *B. bassiana* strain GHA (BotaniGard 22 WP, 2 lb/ac), methomyl (Lannate SP, 0.75 lb/ac), a combination of tolfenpyrad (Torac, 21 fl oz/ac) and methomyl (Lannate SP, 0.75 lb/ac), and an experimental insecticide referred to as NNI-1171 (21 fl oz/ac). Treatments were administered on May 16, May 24, and June 6.

2.2. *M. persicae* and *B. brassicae* on Broccoli

Study was conducted in a commercial broccoli field in Santa Maria in 2012. The field was planted in cultivar Beneforté on July 31. Each plot was 20' long and 64" wide with 5 rows of broccoli replicated four times in a randomized complete block design. Treatments included an untreated control, acetamiprid (Assail 30 SG, 4 oz/ac), *B. bassiana* (BotaniGard 22 WP, 2 lb/ac), tolfenpyrad (Torac, 21 fl oz/ac), an experimental insecticide, pyrifluquinazon (3.2 fl oz/ac), NNI-1171 (21 fl oz/ac), and sulfoxaflor (Sequoia), at two rates (1.5 and 2 fl oz/ac). All treatments were applied using a CO_2-pressurized backpack sprayer with three flat fan nozzles. A spray volume of 50 gal/acre was used for all, but *B. bassiana* which used 100 gal/ac as per the label recommendations. A non-ionic surfactant was added at 0.1% (v/v) concentration for acetamiprid, 0.125% for *B. bassiana*, and 0.25% for the remaining treatments. Treatments were initiated on September 5 and repeated on September 25. The number of *M. persicae* and *B. brassicae* were monitored on five randomly selected plants per plot prior to the first application and 3, 7, and 12 or 13 days after each spray application. On each observation date, sample plants were pulled out and the number of aphids on each leaf was counted. Data were subjected to analysis of variance and significant means were separated using Tukey's HSD test.

3. RESULTS AND DISCUSSION

3.1. *F. occidentalis* On Lettuce

3.1.1. 2011 Study

Average number of *F. occidentalis* gradually increased towards the middle of the observation period and declined thereafter Table **1**. Significant differences ($P < 0.05$) among treatments appeared starting from 3 days after the second spray application. When the average for seven post-treatment sampling dates was considered, spinetoram, methomyl, tolfenpyrad, and the combination of tolfenpyrad and methomyl caused a significant reduction ($P = 0.0001$) in *F. occidentalis* Fig. (**1**). Acetamiprid could not limit population build up.

Table 1. Mean number of *F. occidentalis* per plot in 2011 before and 3, 7, and 11 days after treatment (DAT).

Treatment	Pre-treatment	I Spray 3DAT	I Spray 7DAT	II Spray 3DAT	II Spray 7DAT	II Spray 11DAT	III Spray 3DAT	III Spray 7DAT
Untreated	0.50±0.50	6.75±3.83	7.75±1.88	8.00±1.87a*	17.25±3.59a	6.50±1.04ab	4.75±1.49ab	7.00±1.68ab
Acetamiprid	1.25±1.25	2.75±1.79	4.25±2.01	4.75±0.47ab	15.25±3.75ab	8.75±1.03a	7.25±2.05a	9.50±3.22a
Spinetoram	1.75±0.75	1.75±0.62	4.00±0.91	3.00±1.47b	5.75±1.03bc	3.00±0.91b	2.00±0.91b	2.00±1.08bc
Methomyl	1.25±0.62	1.25±0.25	2.50±0.86	2.50±0.86b	4.00±1.22c	3.25±0.85b	2.00±0.70b	0.75±0.25c
Tolfenpyrad	0.00	1.00±0.57	3.75±0.62	3.50±1.19b	6.00±1.08bc	2.50±1.19b	1.50±0.64b	2.25±1.03bc
Tolfenpyrad+Methomyl	1.25±0.47	1.25±0.62	3.25±0.75	3.50±0.50b	3.75±1.10c	3.50±0.64b	2.00±0.70b	1.00±0.40bc
***P* value**	0.537	0.228	0.061	0.005	0.002	0.001	0.012	0.001

*Means followed by the same or no letter within each column are not significantly different using Tukey's HSD.

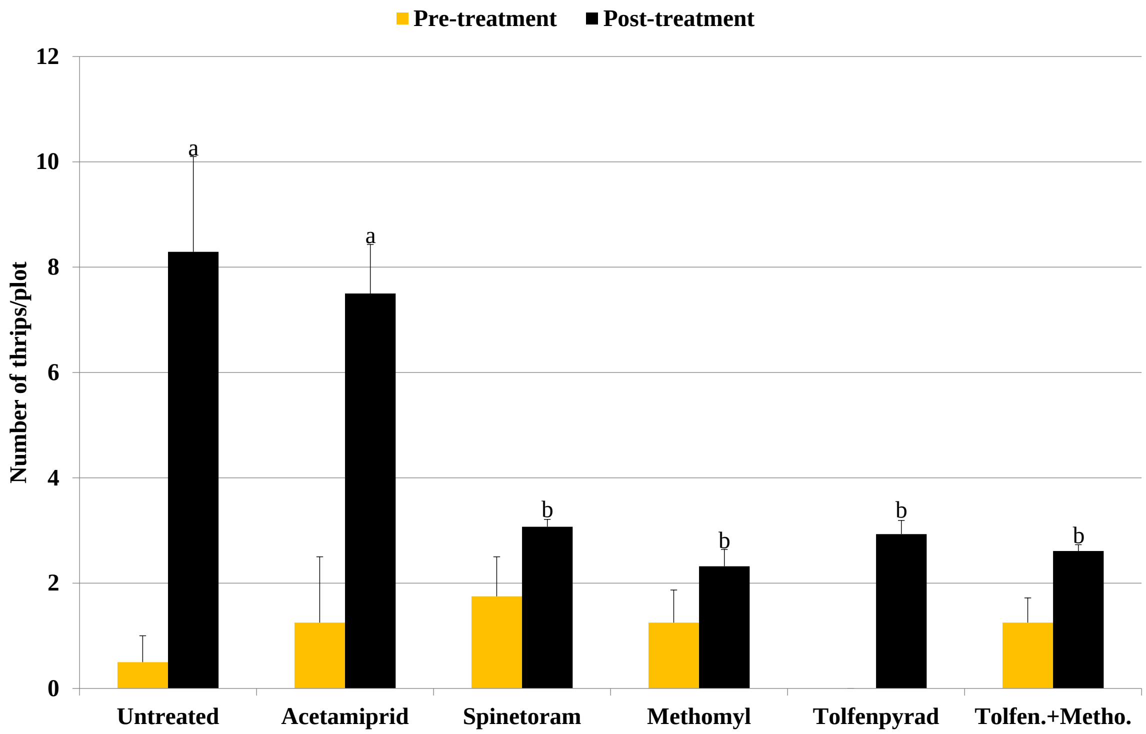

Fig. (1). Number of *F. occidentalis* per plot before and after (average of seven sampling dates) treatment in 2011. Bars with no or same letters are not significantly different using Tukey's HSD ($P \geq 0.05$).

3.1.2. 2012 Study

The number of *F. occidentalis* was similar before the initiation of the experiment with a general decline after the second spray application and an increase thereafter Table (**2**). Significant differences ($P < 0.05$) among treatments were observed 7 and 12 days after the second spray application and 3 days after the third spray application. Post-treatment average during the seven sampling dates showed that tolfenpyrad alone and in combination with methomyl significantly reduced ($P < 0.00001$) *F. occidentalis* numbers compared to untreated control, acetamiprid, and *B. bassiana* Fig. (**2**). Plots treated with spinetoram, *B. bassiana*, and NNI-1171 had moderate levels of *F. occidentalis* during the post-treatment observation period.

Tabel 2. Mean number of *F. occidentalis* per plot in 2012 before and 3, 7, and 12 days after treatment (DAT).

Treatment	Pre-treatment	I Spray 3DAT	I Spray 7DAT	II Spray 3DAT	II Spray 7DAT	II Spray 12DAT	III Spray 3DAT	III Spray 7DAT
Untreated	6.25±3.27	2.50±1.04	14.50±3.40	9.75±0.47	20.00±3.74a*	17.25±2.05ab	11.75±1.88ab	9.25±3.11
Acetamiprid	3.75±0.25	3.50±1.19	14.25±2.71	9.25±2.17	11.75±2.62ab	18.75±3.42a	16.75±4.13a	13.50±3.06
Spinetoram	5.25±1.54	2.25±1.10	8.00±1.58	5.50±1.70	9.00±2.73b	15.75±1.88ab	8.50±1.19ab	7.25±0.94
B. bassiana	5.75±1.25	3.75±0.62	11.25±2.56	6.50±1.50	10.25±1.10ab	12.50±1.32ab	11.75±2.46ab	9.75±2.13
Tolfenpyrad	7.75±1.03	0.50±0.28	7.00±2.27	2.50±0.86	3.25±1.25b	8.25±0.62b	3.50±0.50b	8.75±1.43
Tolfen.+Methomyl	6.25±1.97	1.75±0.62	7.50±1.32	3.75±1.70	9.25±2.68ab	6.75±1.79 b	4.75±1.54ab	6.25±2.25
NNI-1171	5.00±1.68	1.00±0.40	13.00±4.81	6.00±2.27	4.75±2.42b	11.00±4.50ab	4.00±1.82b	3.75±1.43
***P* value**	0.696	0.151	0.235	0.121	0.002	0.025	0.013	0.087

*Means followed by the same or no letter within each column are not significantly different using Tukey's HSD.

There were minor variations in the efficacy of various insecticides from 2011 to 2012, but acetamiprid could not control *F. occidentalis* in both years. Acetamiprid, which was used as a grower standard in the current studies, effectively controlled *F. occidentalis* in laboratory and greenhouse studies on lettuce, pepper, and tomato in an earlier study by Broughton and Herron [18]. Results indicate possible resistance to acetamiprid and suggest a need for rotating insecticides from different modes of action groups both as a means of resistance management and to achieve effective control. Efficacy of *B. bassiana* was similar to spinetoram and NNI-1171 among the remaining treatments. Significant reduction in the numbers of *F. occidentalis* larvae, adult or soil-dwelling stages was seen in different laboratory or greenhouse studies using commercial or local isolates of *B. bassiana* [13, 19, 10, 20, 21]. However, data on the field efficacy of *B. bassiana* in lettuce, especially in California is lacking and the current studies provide useful insights into the understanding of field efficacy of a commonly available entomopathogenic fungus.

3.2. Aphids on Broccoli

M. persicae - All treatments reduced the number of *M. persicae* by the end of the observation period except for acetamiprid Table (**3**). Significant differences among treatments were seen only on 3 ($P = 0.01$) and 12 ($P = 0.02$) days after treatment. When the average for six post-treatment sampling dates was considered, sulfoxaflor at the low rate was the only treatment that had significantly ($P = 0.03$) lower number of *M. persicae* than untreated control Fig. (**3**). When the pre- and post-treatment averages were compared, there was a slight increase in *M. persicae* in acetamiprid and pyrifluquinazon treatments whereas sulfoxaflor high rate caused a 73% reduction followed by sulfoxaflor low rate (47%), NNI-1171 (44%) and *B. bassiana* (40%).

Table 3. Mean number of *M. persicae* per plot in 2012 before and 3, 7, 12 or 13 days after treatment (DAT).

Treatment	Pre-treatment	I Spray 3DAT	I Spray 7DAT	I Spray 13DAT	II Spray 3DAT	II Spray 7DAT	II Spray 12DAT
Untreated	9.75±2.86	6.75±4.09	9.75±2.52	6.50±3.47	13.25±2.01a*	7.50±3.30	9.50±4.21a
Acetamiprid	3.75±2.25	6.75±2.78	4.50±2.59	1.75±1.03	0.75±0.47b	6.50±3.84	6.25±3.11ab
B. bassiana	11.00±6.39	9.25±2.92	10.25±3.37	5.50±2.25	5.00±1.77ab	8.50±4.64	1.00±0.40ab
Tolfenpyrad	7.50±4.17	6.00±2.79	4.25±3.27	8.00±2.08	4.50±3.06ab	3.50±2.17	2.75±1.25ab
Pyrifluquinazon	3.00±2.04	0.50±0.50	6.25±3.56	6.50±4.01	3.25±2.92b	2.00±1.22	0.75±0.47ab
NNI-1171	5.75±2.42	1.50±1.50	2.00±2.00	4.25±2.65	2.25±1.31b	8.75±8.09	0.50±0.50ab
Sulfoxaflor 1.5	2.75±1.25	3.25±1.43	0.75±0.47	4.25±3.59	0.50±0.50b	0	0b
Sulfoxaflor 2.0	9.00±6.12	0.75±0.47	2.75±2.42	2.25±2.25	3.50±3.17b	3.00±2.67	2.50±0.86ab
***P* value**	0.694	0.095	0.075	0.771	0.005	0.703	0.023

*Means followed by the same or no letter within each column are not significantly different using Tukey's HSD.

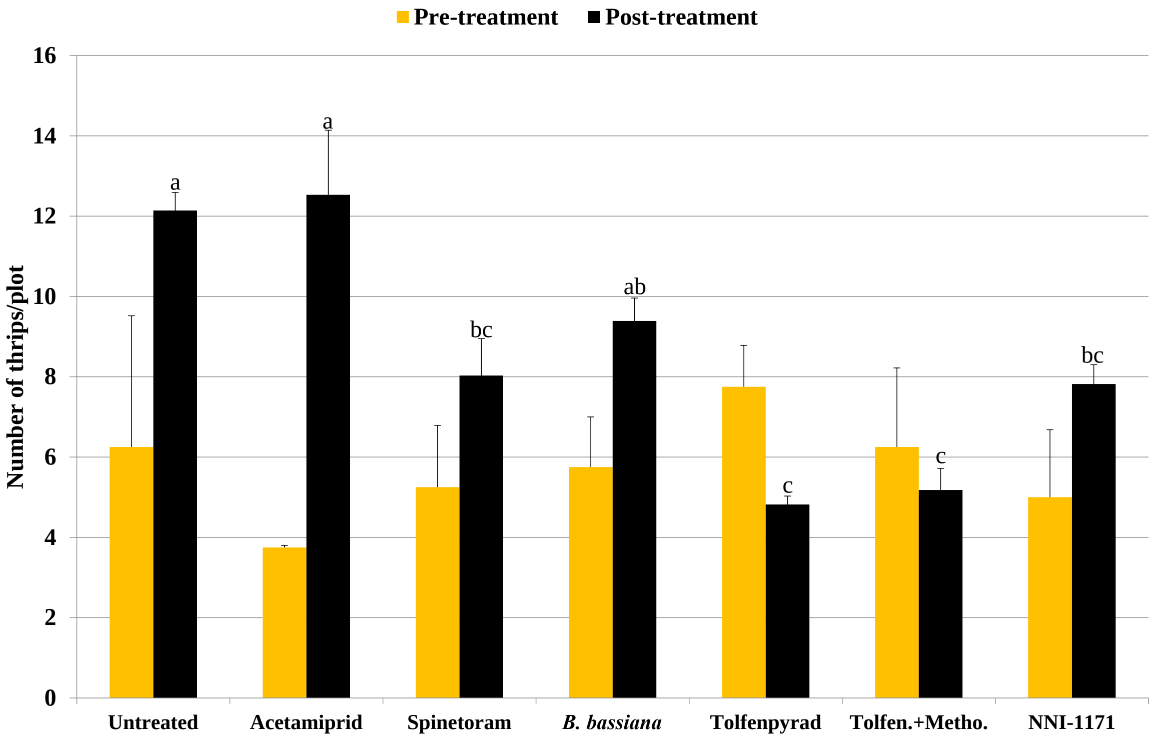

Fig. (2). Number of *F. occidentalis* per plot before and after (average of seven sampling dates) treatment in 2012. Bars with no or same letters are not significantly different using Tukey's HSD ($P \geq 0.05$).

B. brassicae - There was a general reduction in *B. brassicae* numbers in all plots Table (**4**). Significant differences among treatments were seen only on 3 and 7 days after the first spray application ($P = 0.01$). When the post-treatment average was considered, sulfoxaflor and acetamiprid had significantly lower ($P = 0.003$) numbers of *B. brassicae* compared to untreated control Fig. (**4**). When the pre- and post-treatment averages were compared, acetamiprid and sulfoxaflor treatments caused about 90% reduction in *B. brassicae* followed by pyrifluquinazon, which caused about 80% reduction.

Table 4. Mean number of *B. brassicae* per plot in 2012 before and 3, 7, 12 or 13 days after treatment (DAT).

Treatment	Pre-treatment	I Spray 3DAT	I Spray 7DAT	I Spray 13DAT	II Spray 3DAT	II Spray 7DAT	II Spray 12DAT
Untreated	10.00±1.08	5.75±5.10ab*	8.75±2.35a	10.00±4.91	9.50±4.03	4.25±3.61	7.75±3.92
Acetamiprid	14.50±10.71	0.50±0.28b	0.75±0.75b	2.25±1.93	0	3.50±2.36	0
B. bassiana	9.00±1.87	19.50±5.89a	5.75±2.25ab	14.00±3.48	1.50±0.95	1.00±0.57	1.75±1.18
Tolfenpyrad	8.00±4.88	3.50±2.59b	4.75±1.79ab	5.00±2.79	4.00±3.36	0	7.75±4.32
Pyrifluquinazon	9.75±3.75	0b	1.50±0.86ab	2.25±0.75	1.00±1.00	7.25±5.99	0
NNI-1171	2.50±1.65	4.25±3.61ab	0.25±0.25b	2.50±1.32	1.75±1.43	12.25±10.34	0.75±0.75
Sulfoxaflor 1.5	9.75±5.10	0.75±0.47b	2.00±0.81ab	2.25±1.31	1.25±1.25	0	0
Sulfoxaflor 2.0	15.50±3.86	0.50±0.50b	2.25±2.25ab	3.25±3.25	2.75±1.60	0	0.50±0.28
***P* value**	0.579	0.006	0.010	0.048	0.129	0.494	0.050

*Means followed by the same or no letter within each column are not significantly different using Tukey's HSD.

Efficacy of some insecticides varied between species of aphids. In general, sulfoxaflor provided a good control of both species. Acetamiprid and pyrifluquinazon could not limit the increase in *M. persicae*, but caused a significant reduction in *B. brassicae*. On the other hand, NNI-1171 reduced *M. persicae* numbers, but could not control *B. brassicae*. Both *B. bassiana* and tolfenpyrad reduced both species of aphids, but *B. bassiana* was more effective against *M. persicae* with a 40% reduction post-treatment compared to *B. brassicae* with only 19% reduction. Efficacy of *B. bassiana* could vary depending on the isolate and insect species [14]. For example, Ying, Feng, Xu, and Ma [22] reported effective control of *M. persicae* in field cabbage with a Chinese isolate, while Butt, Ibrahim, Ball, and Clark [23] found that European isolates of *B. bassiana* were less effective against *M. persicae* and other pests of Chinese cabbage compared to the Brazilian or European isolates of another entomopathogenic fungus *Metarhizium anisopliae*.

Laboratory assays also showed that *B. bassiana* isolates varied in their pathogenicity to *M. persicae* [24] and *B. brassicae* [25].

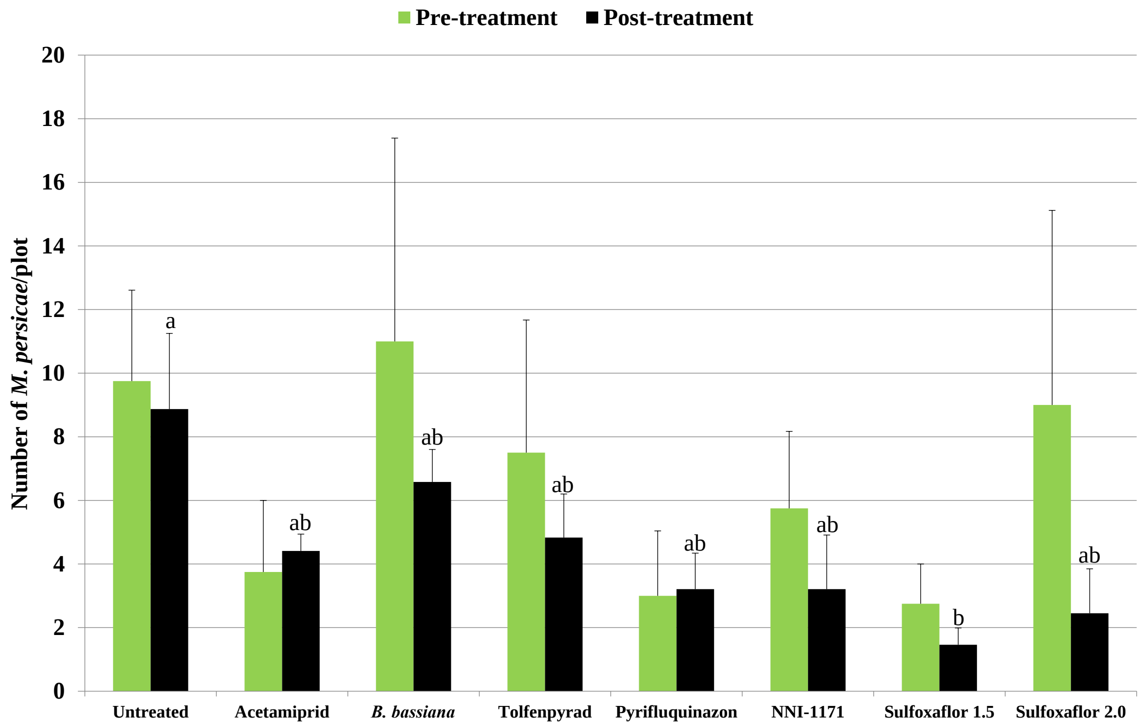

Fig. (3). Number of *M. persicae* per plot before and after (average of six sampling dates) treatment in 2012. Bars with no or same letters are not significantly different using Tukey's HSD ($P \geq 0.05$).

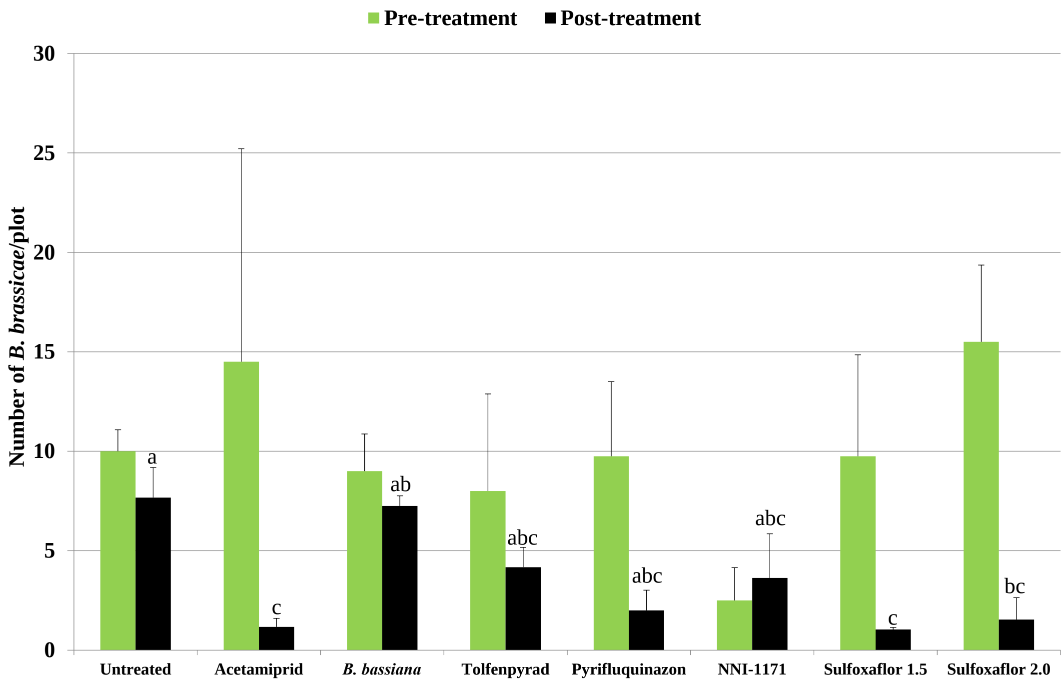

Fig. (4). Number of *B. brassicae* per plot before and after (average of six sampling dates) treatment in 2012. Bars with no or same letters are not significantly different using Tukey's HSD ($P \geq 0.05$).

Since entomopathogenic fungi take time for the infection process, combining *B. bassiana* with other insecticides might improve its efficacy against vegetable pests. Such a positive impact was seen against *F. occidentalis* and other pests in California strawberries when *B. bassiana* was combined with chemical pesticides or azadirachtin [26]. Similarly, root aphids in organic celery were effectively controlled when *B. bassiana* was used in combination with azadirachtin [27].

These studies demonstrate the efficacy of various chemical insecticides and *B. bassiana* against some of the lettuce and broccoli pests and provide useful information for growers. While chemical pesticides are more readily used by growers due to various reasons including low cost and generally perceived higher efficacy, microbial pesticides such as those based on *B. bassiana* are also important for reducing the risk of pesticide resistance and maintaining environmental sustainability. However, limited information is available on microbial control of vegetable pests especially in California. Current studies evaluated the potential of *B. bassiana* in vegetable IPM. In circumstances where microbial pesticides alone are not very effective, combining or rotating them with chemical pesticides or other control options can be effective [28]. Using insecticides with different modes of action along with non-chemical alternatives is critical for sustainable pest management practices.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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